Docket No.: 4518-0111PUS1
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Hans LOIBNER et al.

Application No.: 10/552,324 | Confirmation No.: 8937

Filed: October 7, 2005 | Art Unit: 1643

For: IMMUNOGENIC RECOMBINANT ANTIBODY | Examiner: L.A. Bristol

DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner of Patents
P.O. Box 1450
Alexandria, VA 22313-1450

March, 21st 2011

Sir:

I, Dr. Manfred Schuster COO of Apeiron Biologics AG, Vienna, do hereby declare the following:

I have attached a copy of my curriculum vitae to this Declaration.

I am COO of Apeiron Biologics and have worked in this field for more than 15 years.

I am familiar with the above referenced patent application, as well as the development, usages and properties of recombinant antibodies.

I have read and understand the subject matter of the Office Action of March 24, 2010.

The following comments are offered in support of the patentability of the instant invention.

Summary of Experiment

The following experiment was conducted to demonstrate that one of skill in the art would understand that the presently claimed antibodies and antibody fragments are immunogenic.

Antibodies used in Experiment

The antibodies used in the experiment are recombinant mAb-17A antibodies having a sequence which includes amino acids 30-243 of SEQ ID NO: 4 and amino acids 20-465 of SEQ ID NO: 2, both recited in the Specification and Sequence Listing of the present application. Amino acids 1-29 of SEQ ID NO: 4 and 1-19 of SEQ ID NO: 2 are cleaved off during processing of the expression product (*i.e.* secretion) in mammalian cells as described in the Specification at page 20, line 30 to page 21, line 15.

The N-linked carbohydrate moiety recited in the claims is attached during post-translational processing of the antibody at amino acid 315 of SEQ ID NO: 2. N-linked carbohydrates typically attach to antibodies expressed in mammalian cells at the sequence NST (Jefferis et al., Immunol Lett. 1995).

Outline of Experiment

Antibodies were made and verified according to the processes described in Examples 1-6 of the instant Specification. The present study was conducted as follows: This protocol is similar to that of Example 7 of the Specification.

4 Rhesus monkeys were vaccinated subcutaneously on days 1, 15, 29 and 57 each with 0.5 mg Mab 17-1A adsorbed on aluminum hydroxide, 3 Rhesus monkeys were

vaccinated subcutaneously with 0.5 mg rec Mab 17-1A adsorbed on aluminum hydroxide, using the same schedule. Blood was withdrawn on study days 1, 15, 29, 43, 57 and 71; and serum was obtained for analysis of the immune response against Mab 17-1A or rec Mab 17-1A.

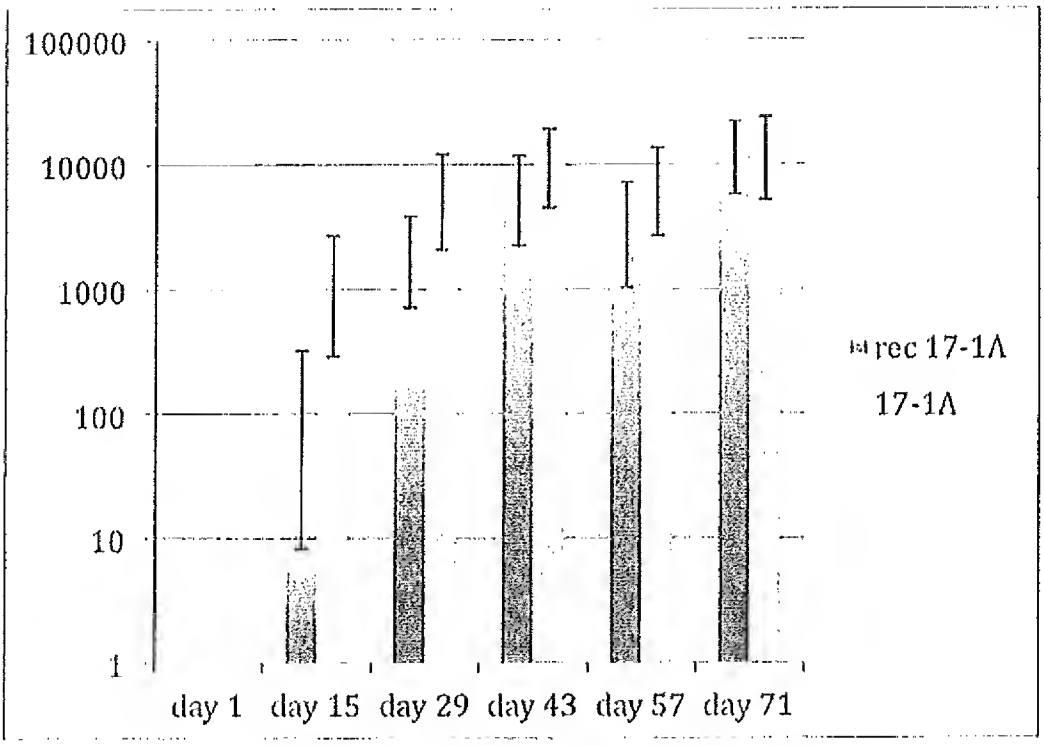
Titers of immunoglobulin (IgG, IgM, IgA) raised against the respective vaccine antigen (Mab 17-1A or rec Mab 17-1A) were measured by Sandwich ELISA using Mab 17-1A or rec Mab 17-1A to coat the plates.

Results of Experiment

As shown in the following figure, vaccination with Mab 17-1A or rec Mab 17-1A as vaccine antigen led to a very similar induction of an immune response, no significant differences were seen. Following four vaccinations, both vaccine antigens induced serum titers of more than 1:10,000.

It is important to note that the antibodies here are being used as immunogenic molecules *per se* and not for their antibody functions. Each antibody molecule is composed of two heavy and two light chains, and contains both linear and three-dimensional immunogenic epitopes. These epitopes can be located both within the constant or the variable murine regions of the antibody, whose sequences differ fundamentally from respective human sequences. Our immune system is trained to recognize such foreign structures, which are recognized by specific T- and B-cells after being processed into peptides by respective antigen presenting cells. The presentation of these linear epitopes elicits as consequence a humoral and cellular immune response. Therefore, it is my opinion that using an antibody fragment comprising the claimed sequences would also be immunogenic, since these sequences and parts of are of murine

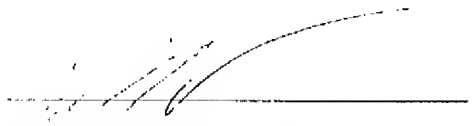
origin and differ therefore from the ones of humans. Especially the nature of this antibody being a murine IgG2a isotype will contribute to its immunogenicity, since the human antibody repertoire does not contain this very special isotype specific for rodent species.



Thus, it is my opinion that one of skill in the art would understand from the present Specification that an antibody or antibody fragment comprising SEQ ID NO: 2 and/or SEQ ID NO: 4 would generate an immunogenic response.

Moreover, one of skill in the art would have understood based on the Jefferis et al. publication, where the non-human mammalian glycosylation would attach and that it would enhance the immunogenicity of the antibody or antibody fragment.

I hereby declare that all statements made herein of my own knowledge are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

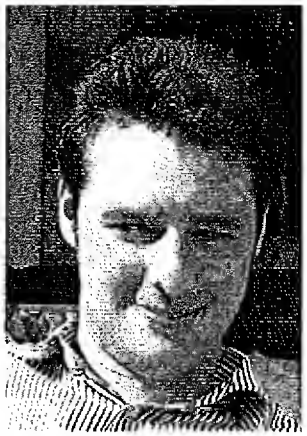


March 21st, 2011

Signed/ Manfred Schuster Date

CURRICULUM VITAE

Manfred Schuster



Personal data

Title	VP Research and Development CSO
Address	Josef -Weilandstrasse 84, A-2191 Schrick +43 (0)2574 2434 (home) +43 (0)664 8405040 (mobile) manfred.schuster@apeiron-biologics.com
Family status	Married (since 1994), 1 child (Born 1995)
Date of birth	August 27, 1972, Vienna, Austria
Nationality	Austria

Education

1978-1990	Lycée Français de Vienne, Baccalauréat 1990, scientific section
1990-1997	University education: Chemistry, Biochemistry, Technical chemistry and Biotechnology, University and Technical University of Vienna, Austria

Degrees

1996	Chemistry and Biochemistry, First Degree, University of Vienna
1997	Biochemistry, Biotechnology Dipl.-Ing. (Master of science engineer) Technical University of Vienna Masters Thesis: **Optimization of an ABE-Fermentation**
2000	Biochemistry, Biotechnology Dr. nat. techn. University of Natural Resources and Applied Life Sciences, Vienna Thesis: **Establishment of a high throughput protein expression system in Yeast**

Qualifications and experience

- CSO and Head of Research and Development at Apeiron Biologics
- Expert in Molecular Biology, focused on protein, enzyme and especially antibody expression in pro- and eukaryotic expression systems, 11 years in practice

- Trained biochemist and biotechnologist with strong expertise in protein chemistry, enzymology and downstream processing
- Trained Project Manager
- Experienced working group leader (up to 6 FTE)
- Industry and drug development experience: 3 years Novartis (Genetics unit), 6 years igeneon (Immunotherapy of Cancer), 2 year CSO at Apeiron Biologics
- "Start up", laboratory and company establishment experience
- Fluent in English, French (Baccalauréat Francais), German

Professional achievements and tasks

09/2005 – actually Apeiron Biologics, Vienna, Austria

- **CSO and Head of Research and Development:**

Responsible for the research and development program of three programs
  - Enzyme substitution / enhancement therapy for lung, cardio-vascular and kidney diseases
  - Endogenous pain therapy
  - Immuno-modulation

Head of laboratory and of the scientific staff, company and laboratory establishment

- **Bio-safety Officer** and head of the bio-safety committee

03/2003 – 08/2005 igeneon Immunotherapy of Cancer, Vienna, Austria

- **Program Manager:** Project-champion and responsible for the preclinical development of a Lewis Y-specific therapeutic monoclonal antibody with enhanced effector functions
- **Head of Molecular Biology**
- **Head of a laboratory working group**
- **Bio-safety officer, member of the Key-personnel and Management**

01/2001 – 03/2003 igeneon Immunotherapy of Cancer, Vienna, Austria

- **Project Leader:** Responsible for the development of the cancer vaccine IGN101: Preclinical research, clinical (GLP) analytics until Phase II, establishment of a GMP production process, GMP production, product analytics, product stability, method development, method validation, clinical analytics, bio-assay development
- **Head of a laboratory working group** comprising five employees responsible for Molecular Biology, Protein Expression, Protein Chemistry, Protein Analytics, Cell Line Development, Cell Culture, Chromatography, Up-scaling and Bio-assay Development (ELISA, BIAcore, FACS, ADCC, CDC, PCR)
- **Member of the company Management**

- **Laboratory-Planning**, arrangement and coordination of the construction project for the actual facilities
- **Bio-safety Officer** and head of the bio-safety committee

03/2000 - 01/2001 igeneon Immunotherapy of Cancer, Vienna, Austria

- **Postdoctoral fellow:** Responsible for recombinant protein expression in pro- and eukaryotic expression systems, development of patent free expression constructs, responsible for assay development and preclinical studies
- **Head of a laboratory working group** comprising two employees

04/1997 - 03/2000 Novartis Research Center, Vienna

- **PhD student:** Genetics Unit, **Establishment of a high throughput protein expression system in Yeast**

04/1996 - 03/1997 Technical University Vienna

- **Diploma student**

Annex 1: Competences

Organization and Management

- Project Management course (Primas, 2004)
- Employee Leadership course (2004)
- cGMP course for biotechnological products (2002)
- Bio-safety Officer and head of the bio-safety committee
- Responsible for the establishment and for the operation of a laboratory and facility monitoring- and alarm-system, technical troubleshooting
- Working Group Leader: scientific administration, supervising, assay -planning, -realization, -interpretation and -documentation
- Establishment and maintenance of laboratory and facility infrastructure
- Contacts to authorities
- Health, safety and environmental protection, establishment of a safety concept

Technical competence

- Qualification for operation, training, development, validation and assessment of listed techniques
- *Molecular Biology:* DNA/RNA techniques (siRNA, Sequencing, PCR, RT-PCR, cloning, expression, ligation, enzymatical digestion, transformation, cDNA production, RNA preparation,...), protein expression in multiple pro- and eukaryotic expression systems, generation of single chain Fv by subtractive panning of a phage library
- *Chromatography and Downstream processing:* HPLC / FPLC, IEX, Affinity chromatography, SEC, RPC, HIC, Generation of chromatography matrices
- *Protein chemistry / Protein analytics / Immunology:* Western Blot, SDS-PAGE, IEF, sequencing, Dot blot, protein characterization, determination of affinity, immuno-precipitation, ELISA, enzymology, labelling, coupling, endotoxin determination (LAL), Luminex, FACS, SPR (BIAcore)
- *Cell culture / Fermentation:* Cultivation of pro- and eukaryotic cell lines, prokaryotic fermentations, eukaryotic fermentations until 10 L
- *Cellular assays:* ADCC, CDC, ELISPOT, cell proliferation

- IT competence: Software package MS-Office (Word, Excel, Powerpoint, Outlook, Internet Explorer), MS Project, Delta-Graph, Corel Draw, GraphPad, Sigma Plot, Sigma Stat, MedCalc, Auto Assembler, Chromas, Gene Runner, SlideWrite, EndNote, Unicorn, Chromeleon,...

Other qualifications

Skilled first aid man (AUVA, 2004), driving license since 1990, lifeguard training, fulfilled military service

Annex 2 – invited speaker at international congresses and symposia

2008, Protein Therapeutics, CHI Conference, San Diego, California, USA
Development of an ACE2 Enzyme Substitution Therapy

2005, Biochromatographietag, Vienna, Austria
Isolierung und Charakterisierung von Isoformen rekombinanter Antikörper

2005, Protein Therapeutics, CHI Conference, San Diego, California, USA
Development in Cancer Immunotherapy: From a murine to a humanized and finally to a glyco-engineered monoclonal antibody with enhanced effector functions

2004, Cancer Immunotherapeutics, CHI Conference, Boston, Massachusetts, USA
Increased effector functions of a monoclonal antibody by glycoform engineering, recent results.

2004, 5th European Symposium on Biochemical Engineering Science, Stuttgart, Germany
Increased effector functions of a monoclonal antibody by glycoform engineering

2003, Antibody Production and Downstream Processing, IBC conference, Basel, Switzerland
Increased effector functions of a monoclonal antibody by glycoform engineering

2002, Äkta User Seminar, Emmendingen, Germany
Automated sequential affinity chromatography

2000, 5th Interlaken Conference on Advances in Production of Recombinant Proteins, Interlaken, Switzerland
Comparison of two high throughput expression strategies in S.cerevisiae

1999, 19th International Symposium on the Separation of Proteins, Peptides and Polynucleotides, Delray Beach, Florida, USA
High throughput protein expression in Yeast; comparison of two expression strategies

1998, 2nd European Symposium on Biochemical Engineering Science, Porto, Portugal
Expression Strategies for Functional Genomics

Annex 3 – Poster Presentations

ASCO 2002
Murine monoclonal antibody 17-1A used as vaccine antigen (IGN101): Direct induction of anti-EpCAM antibodies by vaccination
Manfred Schuster, Hans Loibner, Evelyne Janzek, Gottfried Himmler, Jungbauer Alois, Rainer Hahn, Astrid Dürauer, Hellmut Samonigg

AACR 2002
Qualitative and Quantitative Dissection of the Immune Response to the Cancer Vaccine Candidates IGN101 and IGN301
Guenter Waxenecker, Gottfried Himmler, Manfred Schuster, Thomas Putz, Erich Wasserbauer, Evelyne Janzek, Renate Ohler, Stefan Stranner, Hans Loibner, Hellmut Samonigg

Treatment of Breast Cancer Patients with the Cancer Vaccine IGN101 that Induces an Immune Response against the Pan-Carcinoma Glycoprotein EpCAM
Hellmut Samonigg, Hans Loibner, Manfred Schuster and Gottfried Himmler

ASCO 2003
Phase II trial to explore the influence of concomitant chemotherapy on the immunogenicity of the cancer vaccine IGN101 in patients with epithelial cancers
H. Samonigg, G. Hofmann, T. Bauernhofer, M. Balic, H. Stoeger1 , G. Himmler, M. Schuster, F. Rosenkaimer, F. Grolss, H. Loibner

Murine monoclonal antibody 17-1A used as vaccine antigen (IGN101): Direct induction of anti-EpCAM antibodies by vaccination of cancer patients
Manfred Schuster, Stefan Stranner, Evelyne Janzek, Hans Loibner, Gottfried Himmler, Hellmut Samonigg

Vaccination with alum-adsorbed antibodies against EpCAM directly induces anti-EpCAM antibodies
Manfred Schuster, Hans Loibner, Evelyne Janzek, Gottfried Himmler; Marija Balic, Guenter Hofmann, Hellmut Samonigg

AACR 2003
Lewis Y / EpCAM co-expression in breast cancer is correlated with poor prognosis
Guido Sauter, Manfred Schuster, Gottfried Himmler, Hans Loibner

Eurocancer 2003
Expression d'EpCAM dans différents tissus cancéreux et normaux : valeur pronostique dans le cancer du sein
Sauter G., Schuster M., Himmler G. and Loibner H.

PEACE 2003
Expression of recombinant antibodies using a tri-cistronic expression system
M. Schuster, G. Waxenecker, G. Himmler, I. Froihofer, C. Schwager, R. Ohler and H. Loibner, igeneon

ISBT 2003
ANALYSIS OF THE SPECIFICITY OF THE HUMORAL IMMUNE RESPONSE INDUCED BY CANCER VACCINE IGN101
Manfred Schuster, Gottfried Himmler, Hans Loibner, Irmgard Froihofer, Cornelia Schwager, Helga Klug, Susanne Wiederkum, Alois Jungbauer, Astrid Duerauer and Rainer Hahn

Treatment of Breast Cancer Patients with the Cancer Vaccine IGN101 that Induces an Immune Response against the Pan-Carcinoma Glycoprotein EpCAM
Hellmut Samonigg, Hans Loibner, Marija Balic, Guenter Hofmann, Manfred Schuster and Gottfried Himmler

AACR 2004, ISBT 2004
Increased effector functions of a monoclonal antibody by glycoform engineering
M. Schuster, P. Umana1, P. Brünker1, I. Froihofer, S. Wiederkum, C. Schwager, H. Klug, G.C. Mudde, G. Himmler and H. Loibner

Annex 4 – Publications

Inhibition of Xenograft Tumor Growth and Down-Regulation of ErbB Receptors by an Antibody Directed against Lewis Y Antigen
Hesso Farhan, Christian Schuster, Markus Klinger, Eva Weisz, Günter Waxenecker, Manfred Schuster, Veronika Sexl, Geert C. Mudde, Michael Freissmuth, and Ralf Kircheis; The Journal of Pharmacology and experimental Therapeutics 2006; 319:1–8.

Compensation of endogenous IgG mediated inhibition of antibody-dependent cellular cytotoxicity by glyco-engineering of therapeutic antibodies
Andreas Nechansky, Manfred Schuster, Wolfgang Jost, Petra Siegl, Susanne Wiederkum, Gilbert Gorr, Ralf Kircheis; Molecular Immunology 2006.

Cancer Immunotherapy, review article
Manfred Schuster, Andreas Nechansky, Hans Loibner and Ralf Kircheis; Biotechnology Journal 2006; 1, 138-147.

In vivo glyco-engineered antibody with improved lytic potential produced by an innovative nonmammalian expression system
Manfred Schuster, Wolfgang Jost, Geert C. Mudde, Susanne Wiederkum, Cornelia Schwager, Evelyne Janzek, Friedrich Altmann, Johannes Stadlmann, Christian Stemmer and Gilbert Gorr, Biotechnology Journal 2007

Method for determining humoral response to active immunization with monoclonal antibody against EpCAM using peptide arrays prepared by SPOT technology
Dürauer A., Berger E.,Schuster M., Wasserbauer E., Mudde G., Himmler G., Jungbauer A., Manuscript accepted to J. Immunol. Methods

Expression of recombinant antibodies using a tri-cistronic expression system.
Schuster M, Waxenecker G, Himmler G, Loibner H; Manuscript in preparation

Increased effector functions of a therapeutic monoclonal Le-Y specific antibody by glycoform engineering.
Schuster M, Umana P, Waxenecker G, Wiederkum S, Schwager C, Himmler G, Loibner H, Mudde G.C; Cancer Research 2005; Sep 1;65(17):7934-41.65 (17).

Two novel additives for serum and serum-free media increase activity of in-vitro mammalian cells.
Karlheinz Landauer, Lucia Strommer, Manuela Kainer, Otto Doblhoff-Dier, Manfred Schuster, Gottfried Himmler, Hans Loibner, Günter Waxenecker, submitted to Biotechnology Process

Expression and purification of homogenous proteins in Saccharomyces cerevisiae based on ubiquitin-FLAG fusion.
Einhauer A, Schuster M, Wasserbauer E, Jungbauer A
Protein Expr Purif 2002 Apr 24:3 497-504

Transmembrane-sequence-dependent overexpression and secretion of glycoproteins in Saccharomyces cerevisiae.
Schuster M, Wasserbauer E, Aversa G, Jungbauer A
Protein Expr Purif 2001 Feb 21:1 1-7

High speed immuno-affinity chromatography on supports with gigapores and porous glass.
Schuster M, Wasserbauer E, Neubauer A, Jungbauer A
Bioseparation 2000 9:5 259-68

Protein expression in yeast; comparison of two expression strategies regarding protein maturation.
Schuster M, Einhauer A, Wasserbauer E, Sussenbacher F, Ortner C, Paumann M, Werner G, Jungbauer A
J Biotechnol 2000 Dec 28 84:3 237-48

Short cut of protein purification by integration of cell-disrupture and affinity extraction.
Schuster M, Wasserbauer E, Ortner C, Graumann K, Jungbauer A, Hammerschmid F, Werner G
Bioseparation 2000 9:2 59-67

Protein expression strategies for identification of novel target proteins.
Schuster M, Wasserbauer E, Einhauer A, Ortner C, Jungbauer A, Hammerschmid F, Werner G
J Biomol Screen 2000 Apr 5:2 89-97

Solvent production by Clostridium beijerinckii NRRL B592 growing on different potato media.
Nimcevic D, Schuster M, Gapes JR
Appl Microbiol Biotechnol 1998 Oct 50:4 426-8

Annex 5 – Inventions and Patent applications

1998

Integration of cell-disrupture and affinity extraction as one step procedure for purification of intracellularly expressed proteins / SW 698
Schuster M, Wasserbauer E

2000

Production and use of protein-carbohydrate conjugates / ID 2000-001
Eckert H., Loibner H., Schuster M., Himmler, G Waxenecker G.
Patent number WO 03/097663, May 15, 2002"Multiepitope Vaccine"AT, PCT

2001

EpCAM mimotope vaccine / ID 2001-001
Loibner H., Himmler G., Waxenecker G., Schuster M., Putz T.
Patent number WO 04/091655, Apr 17, 2003-05-14"Recombinant immunogenic antibody"AT, PCT

IgG2a modified immunoglobulin / ID 2001-002
Loibner H., Himmler G., Waxenecker G., Schuster M., Putz T.
Patent number WO 04/091655, Apr 17, 2003"Recombinant immunogenic antibody"AT, PCT

2002

Tricistronic expression product / ID 2002-002

Loibner H., Himmler G., Waxenecker G., Schuster M.
Patent number WO 04/091655, Apr 17, 2003"Recombinant immunogenic antibody"AT
Magnetic beads formulation / ID 2002-003
Himmler G., Loibner H., Schuster M., Wasserbauer E., Eckert H., Doblhoff-Dier O., Kircheis R., Waxenecker G.
Patent number WO 02/080966, Mar 23, 2001"Autovac II"AT, PCT
Multicompartment Electrophoresis / ID 2002-006
Himmler G., Schuster M., Waxenecker G., Wasserbauer E.
EpCAM fragment / ID 2002-011
Loibner H., Himmler G., Schuster M.
A modular ELISA for simultaneous quantitation / ID 2002-021
Nechansky A., Schuster M., Waxenecker G., Himmler G., Mudde G.

2003

Multi-epitope vaccine containing a SialylTn carbohydrate conjugate / ID 2003-003
Kircheis R. Schuster M. Himmler G. Loibner H
Patent number WO 03/097663, May 15, 2003"Multiepitope Vaccine"PCT
EpCAM epitopes / ID 2003-005
Loibner H. Himmler G. Jungbauer A. Wasserbauer E. Schuster M. Hahn R. Dürauer A.
Patent number WO 04/106917, Jun 02, 2003 "Selection of epitopes for immunotherapy" AT
CIM Discs / ID 2003-006
Loibner H. Waxenecker G. Wasserbauer E. Schuster M.
Low dose vaccine / ID 2003-015
Loibner H. Himmler G. Schuster M.
Patent number EP 04450149.2, Jul 2004, "Low dose IGN101", EP
VEGF as target for passive immuno therapy / ID 2003-016
Loibner H. Schuster M. Waxenecker G.

2004

Antibodies with specific glycosylation and antigenic surface structure / ID 2004-005
Waxenecker G Himmler G Loibner H Landauer K Schuster M Kircheis R
Combination therapy passive/active / ID 2004-007
Loibner H Schuster M
Novel Lewis y antibody / ID 2004-009
Schuster M, Himmler G, Waxenecker G, Mudde G, Loibner H, Loidl M, Redl G
Patent number PCT/EP2004/007787, Jul 14, 2004 "Modified glycosylated antibody" PCT
Increase of targeted cytotoxicity / ID 2004-010
Waxenecker G, Kircheis R, Schuster M, Himmler G
US prov.
Atomadsorptionspectrometry / ID 2004-011
Chabicowsky M Obwaller A Schuster M Szolar O

2005

Obesity treatment / ID 2005-001
Schuster M, Nechansky A, Wasserbauer E, Kircheis R

2006

DREAM Inhibitors
Schuster M, Loibner H, Stranner S
Characterization of enzymatic activity in complex matrices
Schuster M, Loibner H, Janzek E
Glycoenginnered antibodies
Schuster M, Gorr G, Nechansky A., Kricheis R.
Ex vivo silencing technology
Loibner H, Schuster M